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Chondrocyte repopulation of the zone of death induced by osteochondral harvest

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SUMMARY

Objective: Harvesting osteochondral grafts results in a zone of chondrocyte death (ZCD) in and around the periphery of the graft, creating a barrier for chondrocytes to migrate to the graft periphery, thus limiting cartilage-to-cartilage healing. The purpose of this study was to repopulate the induced ZCD through the combined effects of collagenase treatment and delivery of a chemotactic agent.

Design: In bovine cartilage, the ZCD induced by the OATS™ osteochondral harvesting system was determined, followed by a corresponding collagenase treatment to penetrate the developed ZCD. The chemotactic potential of platelet derived growth factor (PDGF-bb), insulin-like growth factor I (IGF-I), and basic fibroblast growth factor (bFGF) (2.5–100 ng/mL) was then assessed using a modified Boyden chamber assay to select an appropriate agent to induce chondrocyte migration. Afterwards, the combined effects of collagenase treatment and chondrocyte chemotaxis on the repopulation of an induced ZCD were examined in cartilage explants over a 4-week-period.

Results: The OATS™ osteochondral harvesting system induced a significant ZCD (173 μm, 95% CI: [72–274 μm]) in the grafts. Chondrocyte chemotaxis was induced by all agents investigated at concentrations greater than 25 ng/mL. After 4 weeks in culture, collagenase treatment alone reduced the ZCD by approximately 40% relative to untreated explants. Coupling the collagenase treatment with 25 ng/mL IGF-I reduced the ZCD by approximately 80% relative to untreated explants, and 65% relative to explants treated only with collagenase.

Conclusion: Treating cartilage explants with collagenase and 25 ng/mL IGF-I resulted in a decreased ZCD after a 4-week-period, and increased chondrocyte density within the induced ZCD.

Introduction

Mosaic arthroplasty, or osteochondral transfer, is a cartilage repair strategy that aims to restore the articulating surface in a joint where a chondral and/or osteochondral defect has occurred. This procedure involves the transplantation of healthy, unaffected osteochondral tissue from non-weight bearing regions of the joint into the defect region that has been prepared for graft insertion[1]. The main benefit of mosaic arthroplasty is that it immediately results in filling of the defect region with high-quality hyaline cartilage resulting in good clinical outcomes. In a review of 789 procedures involving osteochondral transfer in the knee, Hangody *et al.*[2] reported good-to-excellent clinical results in 92% of patients. Similarly, in a consecutive series of 52 patients, Jakob *et al.*[3] reported improved knee function in 86% of patients at 2 years post-op.

Despite the relatively good clinical outcomes, some of the major disadvantages of this procedure are donor site morbidity[4,5] and the general inability of the chondral portions of the transplanted grafts to integrate with the surrounding host cartilage following insertion[6]. This lack of integration can ultimately affect long-term outcomes in patients who have undergone mosaic arthroplasty, as gaps between grafts may serve as starting points for cartilage degeneration over time[7]. This effect has been attributed to the zone of chondrocyte death (ZCD) that is generated upon graft harvesting[6], which generates a region of dead tissue following osteochondral graft harvest. The ZCD creates a barrier (extending up to 390 μm into the tissue[6]) that the chondrocytes must migrate through in order to populate the graft periphery for effective cartilage integration to occur[8]. The lack of chondrocyte migration through this region has been thought to be inhibited by the dense extracellular matrix (ECM) of cartilage[9]. For this reason, several studies have investigated the effect of

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ECM-degrading enzymes to facilitate improved chondrocyte migration, such as: chondroitinase ABC[9,10], trypsin[9], collagenase[9,11] and hyaluronidase[9,11,12], and various combinations of these enzymes[12–14]. Of these enzymatic treatments, only collagenase has been shown to improve chondrocyte migration[9,12]. However, while chondrocyte migration is facilitated in cartilage treated with collagenase, this has only been demonstrated in cartilage manipulated with sharp scalpel blades or biopsy punches, which do not induce a significant ZCD relative to an osteochondral harvesting instrument[6,15]. Thus, it is unknown whether chondrocytes are able to migrate through the significant region of acellular cartilage that constitutes the ZCD following a collagenase treatment. Furthermore, the extent to which collagenase is able to penetrate into cartilage relative to the induced ZCD has yet to be investigated.

Chondrocyte migration within cartilage may be facilitated with chemotactic agents. Recently, chondrocyte chemotaxis has been demonstrated in response to several growth factors, including: insulin-like growth factor I (IGF-I)[16], platelet derived growth factor (PDGF-bb)[17–19], and basic fibroblast growth factor (bFGF)[20,21]. However, no previous study has yet looked specifically at inducing chondrocyte chemotaxis within cartilaginous tissue. Thus, the purpose of this study was to repopulate the induced ZCD through the combined effects of collagenase treatment and delivery of a chemotactic agent.

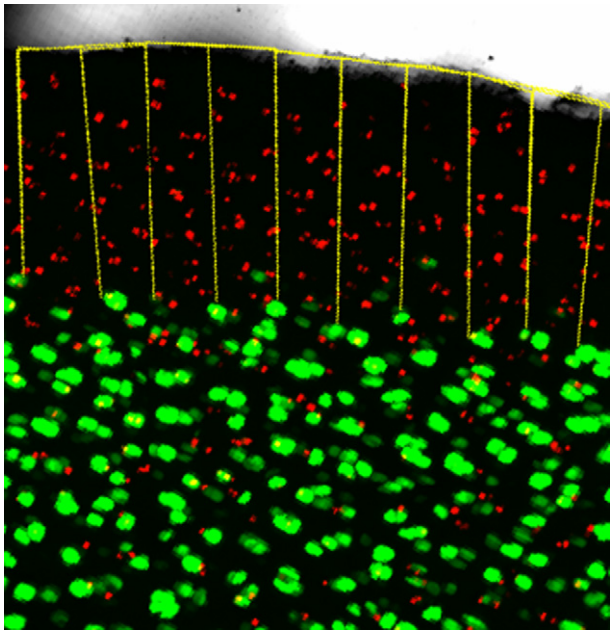

Fig. 1. The extent of the ZCD was determined by measuring the distance from the tissue periphery to the inner region of live cells at several points around each sample.

Materials and methods

Determination of the ZCD

Bovine articular cartilage and bone were harvested from the metacarpal-phalangeal joints of 18 to 24-month-old calves obtained from a local abattoir after slaughter (Brian Quinn's Meats Ltd., Yarker, ON, Canada). Cartilage was trimmed using either a sharp 3 mm biopsy punch (Miltex Inc., York, PA, USA) to simulate scalpel manipulation of cartilage tissue, or the OATS™ osteochondral harvesting system (Arthrex Inc., Naples, FL, USA) to simulate surgical manipulation of the cartilage. The cartilage explants were cultured in Ham's F12 media supplemented with 1 × insulin-transferrin-selenium (ITS^+; Sigma–Aldrich Ltd, Oakville, ON, Canada), 20 mM *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid (HEPES; Sigma–Aldrich Ltd), and antibiotics/antimicotics (100 U/mL penicillin, 100 ug/mL streptomycin, and 0.25 μg/mL amphotericin B; Invitrogen Canada Inc., Burlington, ON, Canada) at 37°C with 5% CO_2 and 95% relative humidity. Explants were cultured for 1 week, with media changed three times per week. Following the culture period, cell viability in the tissues was assessed by using the LIVE/DEAD™ staining kit (Invitrogen Canada Inc.) and viewing under a multiphoton confocal microscope (Leica TCS SP2 with Leica Image Pro Plus software; Leica Microsystems GmbH, Wetzlar, Germany) at 40× magnification. Images were taken at various depths through each tissue sample and images were overlaid to create a projection through the entire depth of the tissue. The extent of the ZCD was determined by measuring the minimum distance from the wound edge at the tissue periphery to the inner region of tissue that contained the first instance of live chondrocytes. Ten measurements were made for each projected image obtained at approximately 50 μm increments along the cut surface, which were then averaged together to quantify the ZCD (Fig. 1).

Collagenase degradation of the periphery of the cartilage explant

Different collagenase treatments were investigated in order to determine the extent of penetration into the tissue. Cartilage explant tissue was harvested, as described previously, and trimmed into 3 mm diameter cylindrical sections using either a biopsy punch or the OATS™ osteochondral harvesting system. Explants were treated with 0.15%, 0.3%, or 0.6% collagenase A (source: *Clostridium histolyticum*; Roche Diagnostics Canada, Laval, QC, Canada) for varying amounts of time (2.5–60 min). Following treatment, the samples were washed thoroughly three times in phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde. The samples were then embedded in paraffin, cut into 5 μm thick sections, and stained with picrosirius red to reveal intact and degraded collagen[22]. Each sample was viewed under light microscopy at 40× magnification, and the depth of the collagenase penetration into the tissue was determined by measuring the distance from the periphery of the tissue to the region of degraded collagen within the tissue with the use of ImageJ software (US National Institutes of Health, Bethesda, MD, USA)[23].

Boyden chamber assays

Primary bovine articular chondrocytes (from the metacarpal-phalangeal joints of 18 to 24-month-old calves) were isolated by sequential enzymatic digestion, as described previously[24]. Chondrocyte chemotaxis was measured using a modified Boyden chamber assay[19,20]. Briefly, 6.5 mm diameter polycarbonate filters with 8 μm pores, (Corning Costar, Lowell, MA, USA) separated the cells seeded on the upper side of the membranes from the chemotactic agent on the underside of the membranes. Prior to cell seeding, the upper and lower compartments were incubated in Ham's F12 media with 0.1% bovine serum albumin (BSA, Sigma–Aldrich Ltd.) at 37°C for 2 h to remove nonspecific protein binding sites. The lower compartments of the filters were then filled with Ham's F12 media supplemented with PDGF-bb, IGF-I, or bFGF (2.5–100 ng/mL; Peprotech Inc., Rocky Hill, NJ, USA), 0.1% BSA, 20 mM HEPES, and antibiotics/antimicotics. The upper compartments were filled with 2×10^5 isolated chondrocytes suspended in Ham's F12 media supplemented with 0.1% BSA, 20 mM HEPES, and antibiotics/antimicotics. Cultures were placed in an incubator maintained at 37°C with 5% CO_2 and 95% relative humidity. After 24 h, chondrocytes remaining on the upper side of the membranes were removed using a cotton swab. Chondrocytes that migrated to the underside of the membrane were fixed in methanol

for 5 min and stained with toluidine blue (Sigma–Aldrich Ltd). Chondrocyte migration was assessed by counting the number of chondrocytes in six random 0.5 mm^2 fields on the underside of each membrane using an inverted light microscope (Hund Wetzlar model Wilovert S; Wetzlar, Germany) with a mounted Leica camera (model DFC320) and ImageJ software[23] at 100× magnification. The average cell counts on the underside of the membrane for each group were normalized to the average cell count on the underside of the membrane for the growth factor-free control group.

Chondrocyte repopulation of the zone of death

Bovine cartilage explant tissue was harvested as described previously. Fresh 3 mm biopsy punches were dulled with the aid of a wooden block so that a ZCD would be generated that is similar to the zone of death that is induced by an osteochondral harvesting instrument[6]. This was done to obtain cartilage samples without underlying subchondral bone as the presence of bone would interfere with the confocal imaging to be conducted on these samples to assess the effect of various treatments to repopulate the ZCD.

Each sample was then assigned to one of the following groups: (1) optimal collagenase treatment to penetrate the ZCD prior to the culture period, (2) optimal collagenase treatment prior to the culture period and delivery of chemotactic agents (PDGF-bb, IGF-I, or bFGF) during the culture period at the optimal concentration to elicit migration, (3) control group with no treatment, or (4) control group with no treatment to be harvested after 10 days (to confirm the extent of the initially induced ZCD which has been shown to take up to 10 days to form[25]). All samples (with the exception of group (4), as noted previously) were cultured for a period of 4 weeks in Ham's F12 media supplemented with 1 × ITS, 20 mM HEPES and antibiotics/antimicotics at 37°C with 5% CO_2 and 95% relative humidity. Following the culture period, samples were treated with the LIVE/DEAD™ staining kit and viewed under a Leica TCS SP2 multiphoton confocal inverted microscope with Leica Image Pro Plus software at 40× magnification. Images were taken at various depths, parallel to the articular surface (at approximately 50 μm steps), through each tissue sample and images were overlaid to create a projection through the entire depth of the tissue. The extent of the ZCD was determined as described previously. The cellular repopulation of the ZCD was semi-quantified by counting the number of live cells within the induced ZCD region of each sample (in the entire overlaid image) normalized to the cellularity of healthy tissue using ImageJ software[23].

Statistical analyses

For experiments involving harvested cartilage or isolated chondrocytes, samples were obtained from a pooled source of dissected legs to reduce inter-animal variability. All numerical results were expressed as a mean ± 95% confidence interval for the mean (95% CI). Experimental and control groups from different conditions were statistically compared using a one-way analysis of variance (ANOVA) with comparisons between individual experimental groups (growth factor concentration, etc.) assessed using Bonferroni-corrected independent sample, *post-hoc t*-tests. All datasets were checked prior to performing statistical tests for both normality and equal-variance with none of the datasets failing either test. Significance was associated with *P*-values less than 0.05 and trends were associated with *P*-values between 0.05 and 0.1. All statistical tests were conducted using SPSS statistical software (SPSS version 16, SPSS Inc., Chicago, IL, USA).

Results

Determination of the ZCD

The extent of the ZCD in tissue samples harvested with the OATS™ osteochondral harvesting system (173 μm, 95% CI: [72–274 μm], $n = 5$) was significantly greater than the extent of the ZCD in samples harvested using a biopsy punch (26 μm, 95% CI: [8–44 μm], $n = 5$) ($P < 0.001$).

Collagenase degradation of the periphery of the cartilage explant

When the OATS™ osteochondral harvesting system was used to harvest the explant tissue, collagenase penetration into the tissue appeared to predominantly progress as a linear front [Fig. 2(A, C, E)]. Conversely, when the explant tissue was harvested using a sharp biopsy punch, collagenase penetration was dependent on the distance from the articular surface of the explants and decreased in a monotonic fashion away from the tissue surface [Fig. 2(B, D, F)]. Regardless of harvesting method, longer treatment times [Fig. 2(C and D)] and higher collagenase concentrations [Fig. 2(E and F)] resulted in greater collagenase penetration into the explants. The optimal collagenase treatment to be used in all subsequent experiments was selected as 0.6% for 10 min as this resulted in the depth of penetration of approximately 175 μm, which corresponded to the extent of the ZCD induced by the OATS™ osteochondral harvesting system observed previously.

Boyden chamber assays

Using a modified Boyden chamber assay, the chemotactic response of chondrocytes to PDGF-bb, bGFG, and IGF-I was assessed over a wide concentration range (2.5–100 ng/mL). In response to the growth factors, increased chondrocyte chemotaxis towards PDGF-bb, bFGF, and IGF-I was observed at a concentration of 25 ng/mL ($P < 0.02$), with no differential effects observed between growth factors (Fig. 3). Similarly, at concentrations above 25 ng/mL, there was no additional effect on chondrocyte chemotaxis for any of the growth factors investigated. For this reason, a growth factor concentration of 25 ng/mL was used to promote chondrocyte chemotaxis in all subsequent experiments.

Overcoming the ZCD

Harvested bovine cartilage explants were maintained *in vitro* for a period of 4 weeks with or without treatment with collagenase or chemotactic agents (Fig. 4). Throughout the culture period, no chondrocytes were observed resident at the periphery of the explants or growing on the bottom of the culture dish. In the untreated explants, the induced ZCD was 179 μm (95% CI: [67–291 μm], $n = 5$) following 10 days in culture and did not significantly change throughout the 4-week-culture period (ZCD at 4 weeks: 175 μm (95% CI: [82–268 μm], $n = 5$)) (Fig. 5). However, each of the treatments had a significant effect on reducing the ZCD relative to the untreated (control) explants ($P < 0.02$, $n = 5$). Explants treated with collagenase and supplemented with bFGF or PDGF-bb did not have a significantly reduced ZCD relative to the collagenase-only treated group ($P = 0.2$, $n = 5$) (Fig. 5). Alternatively, explants treated with collagenase and IGF-I had a significantly reduced ZCD relative to the collagenase-only treated group ($P < 0.001$, $n = 5$).

The cellularity within the ZCD region following 4 weeks in culture was significantly higher in all treatment groups relative to the untreated (control) explants ($P < 0.001$, $n = 5$) (Fig. 6). Explants treated with collagenase and bFGF or PDFG-bb did not have a significantly increased cellularity relative to the collagenase-only

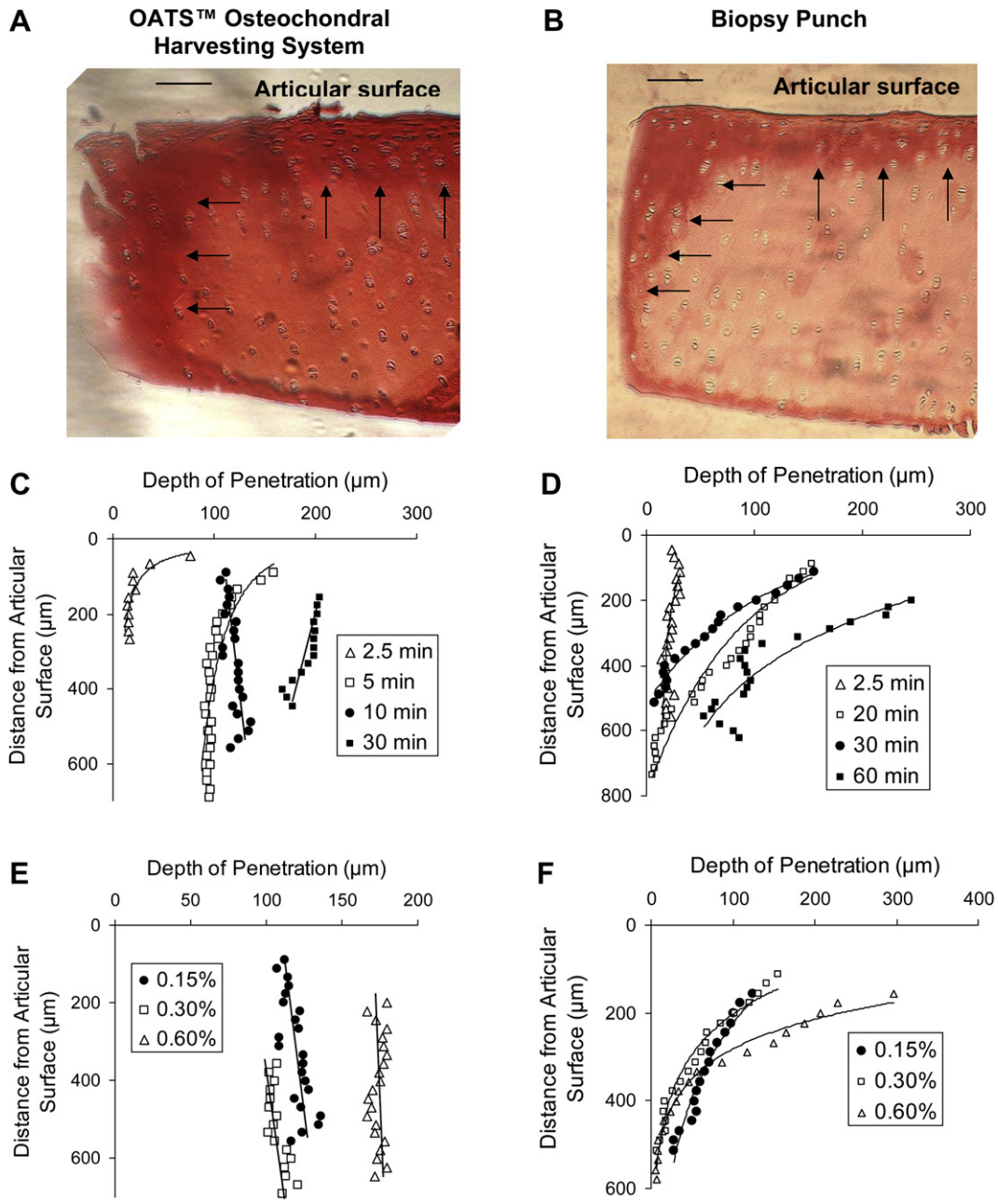

Fig. 2. Collagenase penetration into the wound edge of bovine articular cartilage harvested using either the osteochondral autograft transfer system (OATS) (A, C, E), or a sharp biopsy punch (B, D, F). In sections stained with picrosirius red (A, B), the more intensely-stained regions (indicated by arrows) indicate the depth to which collagenase penetrated into the tissue following 30 min treatment with 0.15% collagenase (magnification 40×, scale bar = 100 μm). The effect of collagenase treatment time (C, D) was assessed at a concentration of 0.15% and the effect of collagenase concentration (E, F) was assessed after an exposure time of 10 min.

treated samples ($P = 0.2$, $n = 5$). However, only explants treated with collagenase and IGF-I had a significantly increased cellularity relative to the collagenase-only treated group ($P < 0.03$, $n = 5$).

Discussion

The purpose of this study was to investigate the combined effects of collagenase treatment and chemotactic agents on the repopulation of the induced ZCD created during osteochondral harvesting. Previous studies have found a significant effect of the harvesting tools on cell death in areas adjacent to the wound edge. In one study, the ZCD created using a surgical osteotome was on the order of 390 μm, whereas a scalpel harvesting resulted in a ZCD of approximately 35 μm[6]. Another study, while not explicitly measuring the induced ZCD, found that cartilage harvested with a blunt instrument resulted in significant cell death at the wound edge, whereas cartilage harvested with a sharp scalpel did not[15]. Some effort has been placed on redesigning osteochondral harvesting tools to minimize the induced ZCD[26]. In this study, while the geometry of the cutting-tip can influence the ZCD, the improved design produced a ZCD of around 120 μm. The results of the present study confirm these findings with osteochondral harvesting tools resulting in significant cell death adjacent to the wound edge and scalpel harvesting resulting in little cell death.

In order to promote chondrocyte outgrowth from cartilage[9] or to increase chondrocyte density at the periphery of cartilage[11,12,14],

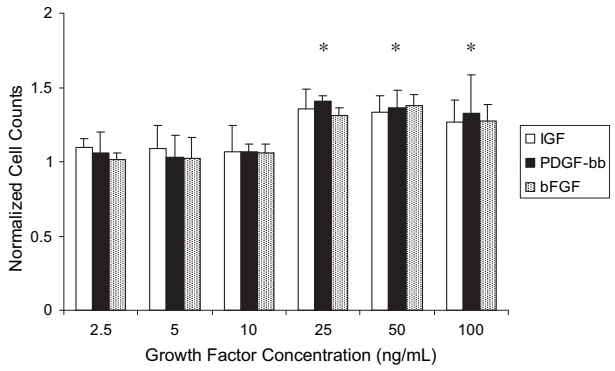

Fig. 3. Chemotaxis dose response of bovine articular chondrocytes to IGF-I, PDGF-bb, and bFGF normalized to non-supplemented controls. Data expressed as mean with 95% CIs ($n = 3$). * Significantly different than control ($P < 0.02$).

several studies have investigated the effect of ECM-degrading enzymes. While pre-treatment with collagenase can be effective to promote chondrocyte migration to the wound edge, the penetration profile into the tissue has not been examined in detail. It is important not to over-treat the tissue as it may degrade more ECM than is necessary, potentially resulting in decreased structural integrity of the osteochondral core. For this reason, in this study we tailored the collagenase treatment to only penetrate the induced ZCD to preserve the structural integrity of the remainder of the explant. One unexpected finding here was that the extent of collagenase penetration appeared to be dependent on the method of harvest. When the explant was harvested with a sharp biopsy punch, the depth of collagenase penetration appeared to be greatest at the articular surface and decreased in a monotonic fashion towards the deep zone of the tissue. However, extracting cartilage explants using the OATS™ osteochondral harvesting created a near uniform depth of penetration throughout the thickness of the explant. While the effect on cartilage-to-cartilage healing following osteochondral implantation was not investigated here, this finding may have significant implications on subsequent tissue integration upon transplantation. The development of a uniform front of outgrowing chondrocytes throughout the thickness of the explant would potentially lead to better integration into the surrounding host cartilage. Additionally, as deep zone chondrocytes are primarily responsible for generating cartilaginous ECM following outgrowth from cartilage explants[27], the penetration of this zone by collagenase after use of an osteochondral harvesting tool would facilitate deep zone chondrocytes to migrate to the tissue periphery and potentially ensure a better healing response. It should be noted that the method used to semi-quantify the depth of collagenase penetration was assessed through histological staining. While picrosirius red is effective at staining both intact and degraded collagen[22], there are some limitations of this approach. Staining intensity can also reflect changes in collagen fibre density. Moreover, the potential loss of glycosaminoglycans from the tissue as a result of collagenase digestion could also potentially lead to more effective collagen staining (due to an unmasking effect). These limitations aside, comparison of the staining between harvesting techniques suggests that the OATS™ osteochondral harvesting system results in a more uniform depth of penetration into the tissue, potentially leading to improved chondrocyte migration towards the wound edge.

In this study, the same chemotactic response was observed in response to each of the growth factors investigated (IGF-I, PDGF-bb, and bFGF) at the same relative concentrations. While there have

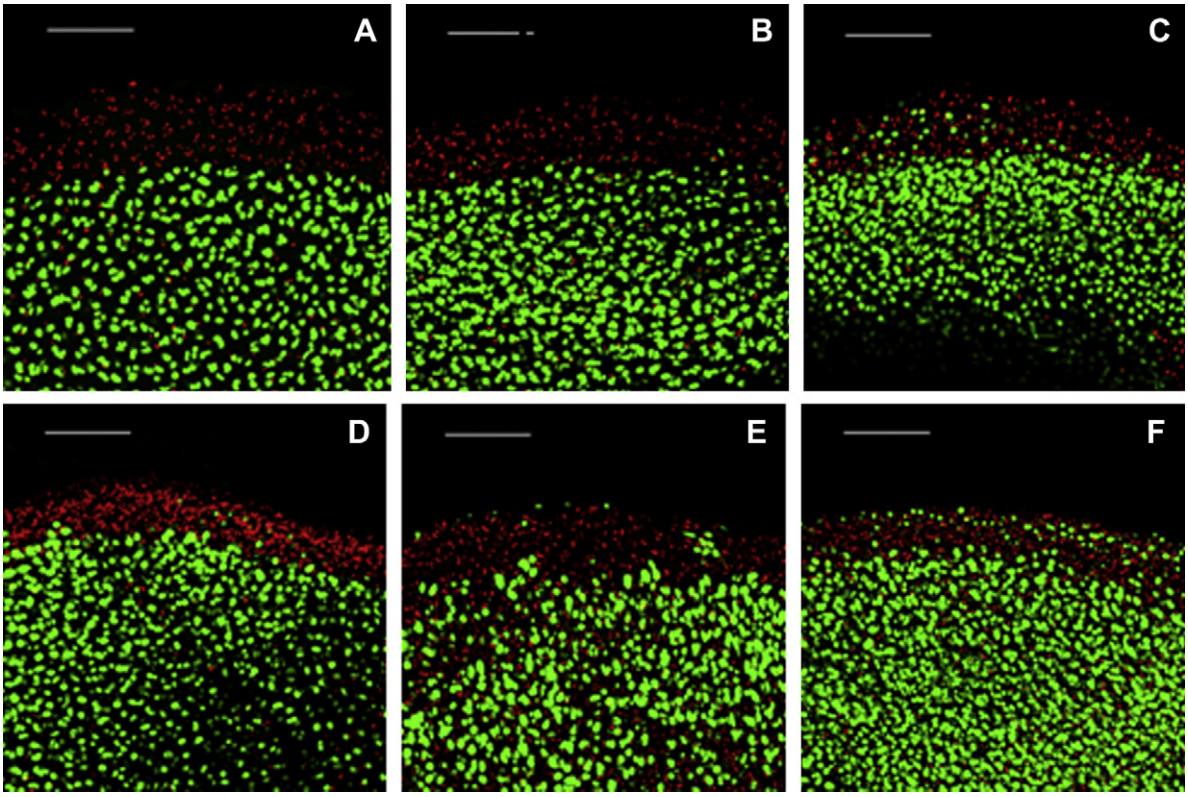

Fig. 4. Confocal images of the effect of various treatments on the repopulation of the ZCD. (A) Initial ZCD after 10 days of culture compared to different treatments after 4 weeks of culture: (B) untreated (control), (C) collagenase treatment only (0.6% for 10 min), (D) collagenase treatment and PDGF-bb (25 ng/mL), (E) collagenase treatment and bFGF (25 ng/mL), and (F) collagenase treatment and IGF-1 (25 ng/mL). Magnification of 40×; scale bar = 200 μm.

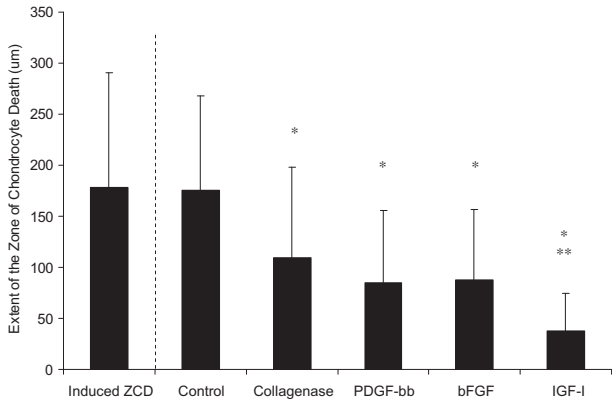

Fig. 5. ZCD measured from the cut surface of bovine articular cartilage explants following a 10-minute treatment with 0.6% collagenase, or collagenase + a chemotactic growth factor (PDGF-bb, bFGF, or IGF-I) at 25 ng/mL. Data expressed as mean with 95% CIs ($n = 5$). * Significantly different than control ($P < 0.02$); ** Significantly different than collagenase-only treatment ($P < 0.001$).

been relatively few studies on chondrocyte chemotaxis in response to growth factors[16–21], there appears to a wide variability in the observed response to these agents. For example, Chang *et al.*[16] found 10 ng/mL IGF-I elicited a two-fold increase in chondrocyte chemotaxis, whereas in the present study, no chemotactic effect was observed at this concentration. Similarly, Maniwa *et al.*[21] observed that bFGF at concentrations greater than 1 ng/mL significantly induced more chondrocyte chemotaxis than lower concentrations, whereas higher threshold responses were observed both in the present study (25 ng/mL) and that by Hidaka *et al.*[20] (5 ng/mL). In contrast, the only agreement between studies was observed in response to PDGF-bb. Mishima *et al.*[19] observed an approximate 60% improvement in chondrocyte chemotaxis at 50 ng/mL PDGF-bb, which is comparable to the present study, in which 50 ng/mL PDGF-bb resulted in approximately 45% more chemotaxis relative to control. While the reason for this wide discrepancy in published results is not explicitly known, there are several potential factors, most notably the difference in species, cell type (primary cells, passage cells or immortalized cell lines) and methodological differences between the studies (e.g., Boyden chambers coatings, timeframe allowed for chemotaxis). Such differences highlight the need for a standardized method for determining chondrocyte chemotaxis to ensure the comparison of results in future studies.

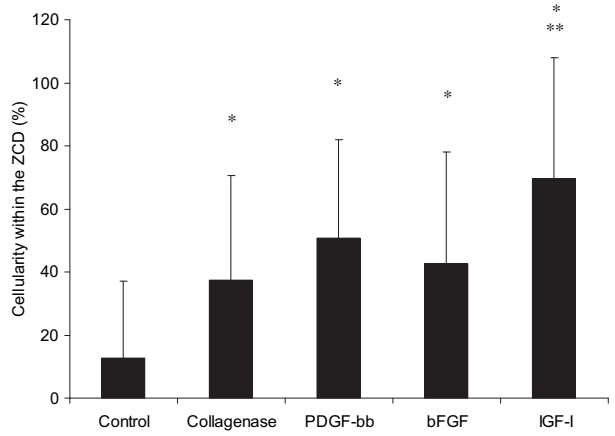

Fig. 6. Cellularity within the initially induced ZCD following 4 weeks in culture, normalized to the cellularity of healthy tissue. Data expressed as mean with 95% CIs ($n = 5$). * Significantly different than control ($P < 0.04$); ** Significantly different than collagenase-only treatment ($P < 0.003$).

Long-term culture of harvested bovine cartilage explants pretreated with collagenase in the presence of chemotactic agents resulted in significant repopulation of the induced ZCD by chondrocytes. This effect was most notable with IGF-I as opposed to either bFGF or PDGF-bb. While it is presently unknown why long-term treatment with IGF-I resulted in a greater degree of ZCD repopulation relative to the other chemotactic growth factors investigated, there may be potential differences in cell proliferation in response to growth factor stimulation over the long-term that could also influence the extent of ZCD repopulation. Irrespective of the exact mechanism of ZCD repopulation, this finding has significant implications to osteochondral transfer as the ZCD represents an area of what is essentially dead cartilage that may disintegrate over time[28]. However, by increasing the cellularity within this region of dead cartilage, the viability may be restored, thus allowing the tissue to remain healthy and potentially leading to better long-term clinical outcomes. Another implication of this work is that if chondrocyte migration into the ZCD continues beyond 4 weeks, it may be possible to encourage the chondrocytes to migrate beyond the periphery of the tissue. Outgrown chondrocytes could have the potential to facilitate cartilage-to-cartilage healing, or fill the gap regions between osteochondral grafts following mosaic arthroplasty with hyaline cartilage. In order to allow for such migration to occur *in vivo*, an appropriate growth factor delivery vehicle must be developed that can deliver a chemotactic agent so that chemotaxis can be sustained.

In this study, it has been demonstrated that chondrocytes are able to repopulate the ZCD induced upon osteochondral harvest following a brief collagenase treatment and supplementation with IGF-I. Clinical implications of ZCD repopulation are the increased long-term viability of the cartilaginous portions of osteochondral grafts and potential to facilitate integration between grafts and surrounding tissue. However, the efficacy of this approach requires a sustained delivery of chemotactic agents, thus requiring the development of a suitable delivery vehicle which is the focus of our continuing research in this area.

Author contributions

Aaron J. McGregor: Conception and design, analysis and interpretation of the data, drafting of the article, collection and assembling of data.

Stephen D. Waldman: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining funding.

Brian G. Amsden: Conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content, final approval of the article, obtaining funding.

Role of the funding source

The funding source provided complete funding for the project (graduate student stipends and directs costs of the research).

Conflict of interest

The authors have no conflicts of interest to declare.

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References

1. Hangody L, Kish G, Karpati Z, Eberhart R. Osteochondral plugs: autogenous osteochondral mosaicplasty for the treatment of focal chondral and osteochondral articular defects. Oper Tech Orthop 1997;7:312–22.
2. Hangody L, Vasarhelyi G, Hangody LR, Sukosd Z, Tibay G, Bartha L, *et al*. Autologous osteochondral grafting – technique and long-term results. Injury 2008;39S1:S32–9.
3. Jakob RP, Franz T, Gautier E, Mainil-Varlet P. Autologous osteochondral grafting in the knee: indication, results, and reflections. Clin Orthop 2002;400:170–84.
4. Reddy S, Pedowitz D, Parekh SG, Sennett BJ, Okereke E. The morbidity associated with osteochondral harvest from asymptomatic knees for the treatment of osteochondral lesions of the talus. Am J Sports Med 2007;35:80–5.
5. LaPrade RF, Botker JC. Donor site morbidity after osteochondral autograft transfer procedures. Arthroscopy 2004;20: e69–73.
6. Huntley JS, Bush PG, McBirnie JM, Simpson AH, Hall AC. Chondrocyte death associated with human femoral osteochondral harvest as performed for mosaicplasty. J Bone Joint Surg 2005;87:351–60.
7. Horas U, Pelinkovic D, Herr G, Aigner T, Schnettler R. Autologous chondrocyte implantation and osteochondral cylinder transplantation in cartilage repair of the knee joint. A prospective, comparative trial. J Bone Joint Surg Am 2003;5:185–92.
8. Morales TI. Chondrocyte moves: clever strategies? Osteoarthritis Cart 2007;15:347–66.
9. Qiu W, Murray MM, Shortkroff S, Lee CR, Martin SD, Spector M. Outgrowth of chondrocytes from human articular cartilage explants and expression of α-smooth muscle actin. Wound Rep Reg 2000;8:383–91.
10. Hunziker EB, Kapfinger E, Muller ME. Removal of proteoglycans from the surface of defects in articular cartilage transiently enhances coverage by repair cells. J Bone Joint Surg 1998;80B:144–50.
11. Bos PK, DeGroot J, Budde M, Verhaar JAN, van Osch GJVM. Specific enzymatic treatment of bovine and human articular cartilage: implications for integrative cartilage repair. Arthritis Rheum 2002;46:976–85.
12. Janssen LM, In der Maur CD, Bos PK, Hardillo JA, van Osch GJVM. Short duration enzymatic treatment promotes integration of a cartilage graft in a defect. Ann Otol Rhinol Laryngol 2006;115:461–8.
13. Quinn TM, Hunziker EB. Controlled enzymatic matrix degradation for integrative cartilage repair: effects on viable cell density and proteoglycan deposition. Tissue Engineering 2002;8:799–806.
14. van de Breevaart Bravenboer J, In der Maur C, Bos PK, Feenstra L, Verhaar J, Weinans H, *et al*. Improved cartilage integration and interfacial strength after enzymatic treatment in a cartilage transplantation model. Arthritis Res Ther 2004;6:R469–76.
15. Redman SN, Dowthwaite GP, Thomson BM, Archer CW. The cellular responses of articular cartilage to sharp and blunt trauma. Osteoarthritis Cart 2004;12:106–16.
16. Chang C, Lauffenburger DA, Morales TI. Motile chondrocytes from newborn calf: migration properties and synthesis of collagen II. Osteoarthritis Cart 2003;11:603–12.
17. Fujita T, Azuma Y, Fukuyama R, Hattori Y, Yoshida C, Koida M, *et al*. Runx2 induces osteoblast and chondrocyte differentiation and enhances their migration by coupling with PI3K-akt signalling. J Cell Biol 2004;166:85–95.
18. Fukuyama R, Fujita T, Azuma Y, Hirano A, Nakamuta H, Koida M, *et al*. Statins inhibit osteoblast migration by inhibiting rac–akt signalling. Biochem Biophys Res Commun 2004;315:636–42.
19. Mishima Y, Lotz M. Chemotaxis of human articular chondrocytes and mesenchymal stem cells. J Orthop Res 2008;26:1407–12.
20. Hidaka C, Cheng C, Alexandre D, Bhargava M, Torzilli PA. Maturational differences in superficial and deep zone articular chondrocytes. Cell Tissue Res 2006;323:127–35.
21. Maniwa S, Ochi M, Motomura T, Nishikori T, Chen J, Naora H. Effects of hyaluronic acid and basic fibroblast growth factor on motility of chondrocytes and synovial cells in culture. Acta Orthop Scand 2001;72:299–303.
22. Van Vinardell T, Dejica V, Poole AR, Mort JS, Richard H, Laverty S. Evidence to suggest that cathepsin K degrades articular cartilage in naturally occurring equine osteoarthritis. Osteoarthritis Cartilage 2009;17:375–83.
23. Rasaband W. ImageJ, 1.42q. United States National Institutes of Health 2005. Available from: http://rsb.info.nih.gov/ij/
24. Waldman SD, Grynpas MD, Pilliar RM, Kandel RA. Characterization of cartilagenous tissue formed on calcium phosphate substrates in vitro. J Biomed Mater Res 2002;62:323–30.
25. Tew SR, Kwan APL, Hann A, Thomson BM, Archer CW. The reactions of articular cartilage to experimental wounding: role of apoptosis. Arthritis Rheum 2000;43:215–25.
26. Huntley JS, McBirnie JM, Simpson AH, Hall AC. Cutting-edge design to improve cell viability in osteochondral grafts. Osteoarthritis Cart 2005;13:665–71.
27. Bos PK, Kops N, Verhaar JAN, van Osch GJVM. Cellular origin of neocartilage formed at wound edges of articular cartilage in a tissue culture experiment. Osteoarthritis Cart 2008;16:204–11.
28. Wakitani S, Goto T, Pineda SJ, Randell YG, Mansour JM, Caplan AI, *et al*. Mesenchymal cell based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg 1994;76A:579–92.